# 1 beetle + 1 beetle =

- Since November 2004 we have produced (with some help from the beetles) over 8,600 individual beetles!
- Beetles are housed individually and kinship is noted.

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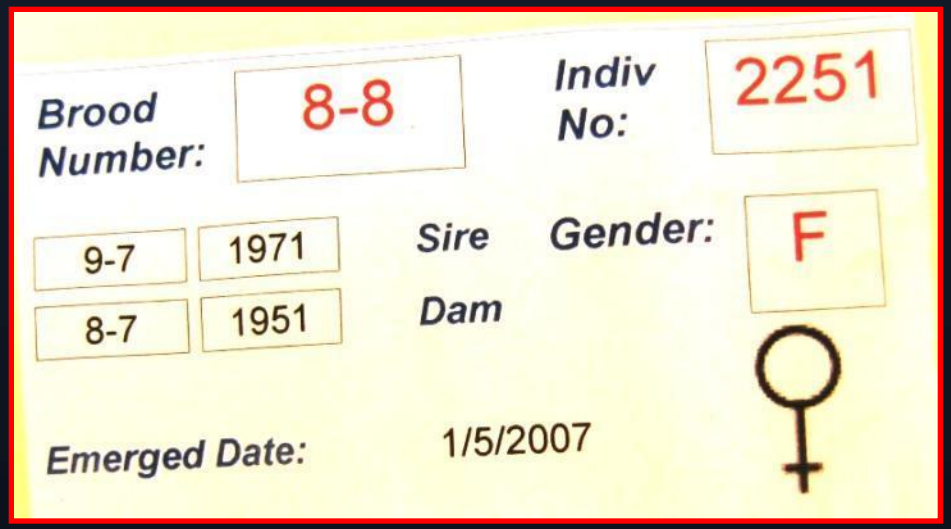
# Individual Records

- We wrote an Access Database program that tracks every beetle.
- Program assigns individual numbers and allows entry of various data fields.

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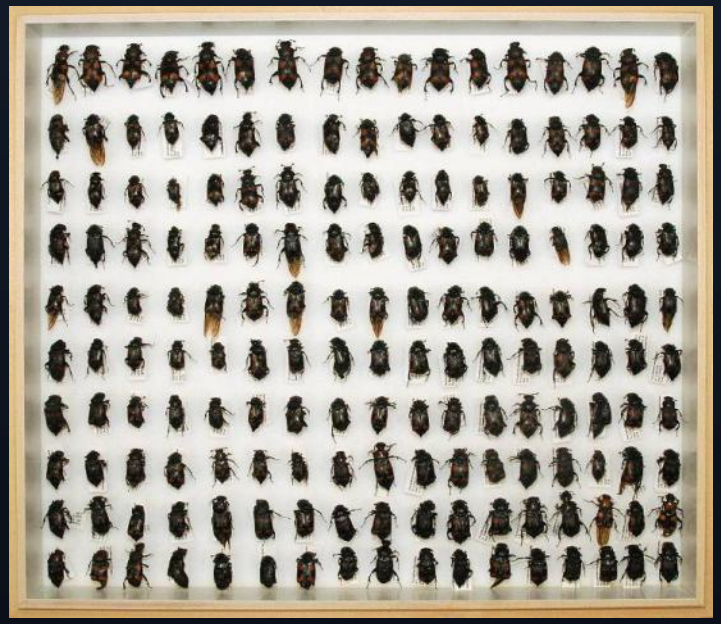
# Record keeping

- It even prints pinning labels after death.



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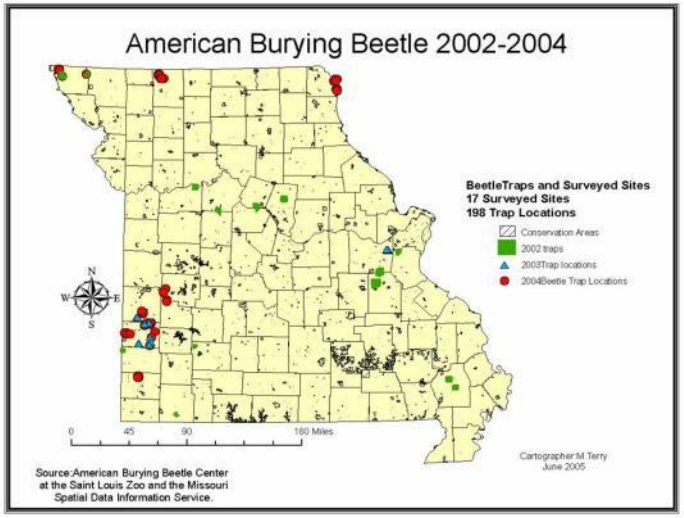
# Survey Totals

- 2002-2013 Totals

- ***Trap nights*** **12,451**
- ***Locations*** **61**
- ***Counties*** **32**
- ***Traps*** **2,244**

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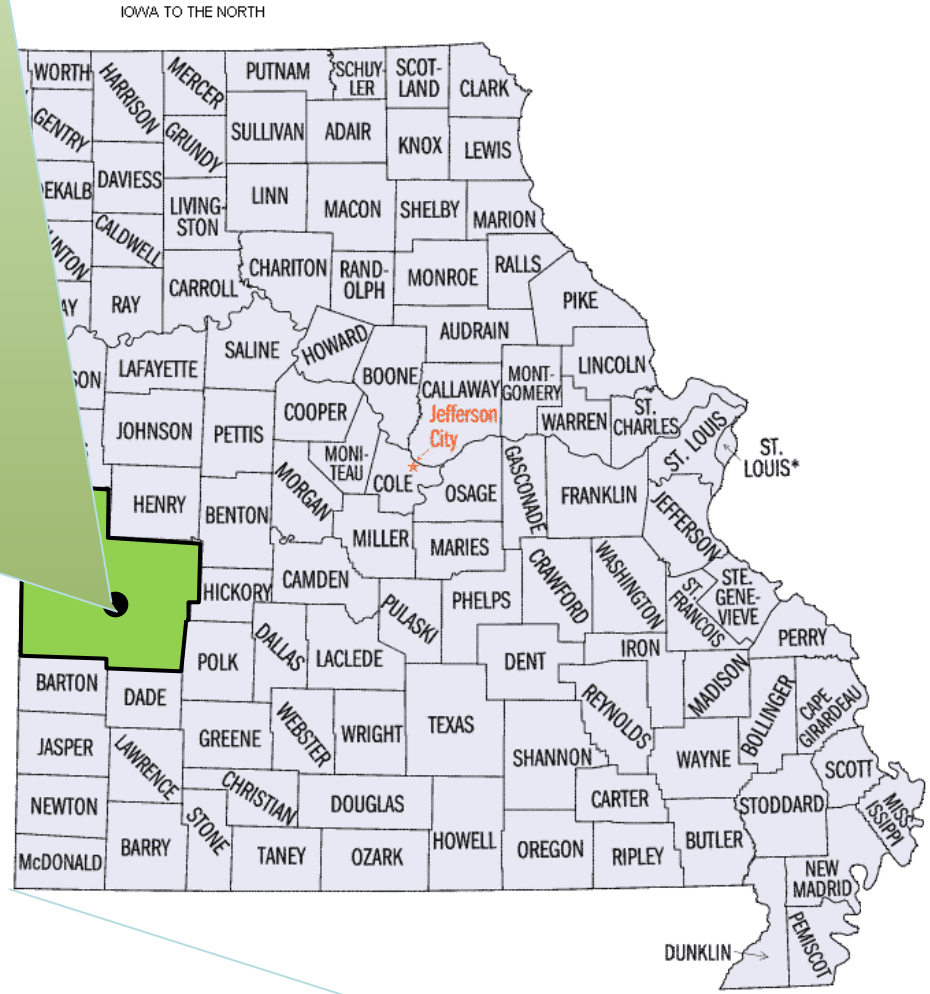
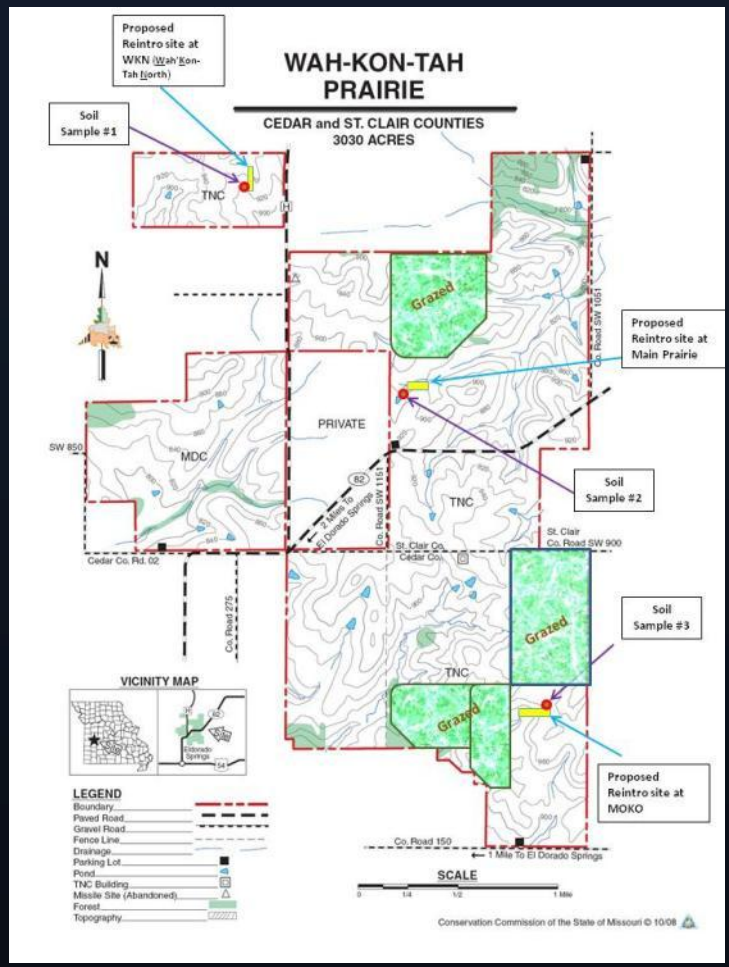
# Nonessential Experimental Population Boundaries



# First Reintro to Missouri

- **June 5, 2012 ….**
- **At Wah' Kon-Tah Prairie.**
  - Site is owned by both TNC and MDC and managed by MDC.

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# Why Wah' Kon-Tah?

- Largest Prairie landscape left in in Missouri
- Well-managed 4,040 acre site
- Donated use of house for surveys
- Greater Prairie Chickens reintroduced there

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# Why Wah’ Kon-Tah?

- Started surveying annually in 2004.
- 2004 - 2012 we had 4,133 trap nights logged.


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# Why Wah' Kon-Tah?

- Five years prior to reintros we captured and released:
- 80,961 Silphids
- 30,602 Nicrophorus

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# Our Reintro method…

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# Reintro method…

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# Reintro method…

- Ten days after reintroduction 30% of broods are randomly checked to ascertain success rate of broods.


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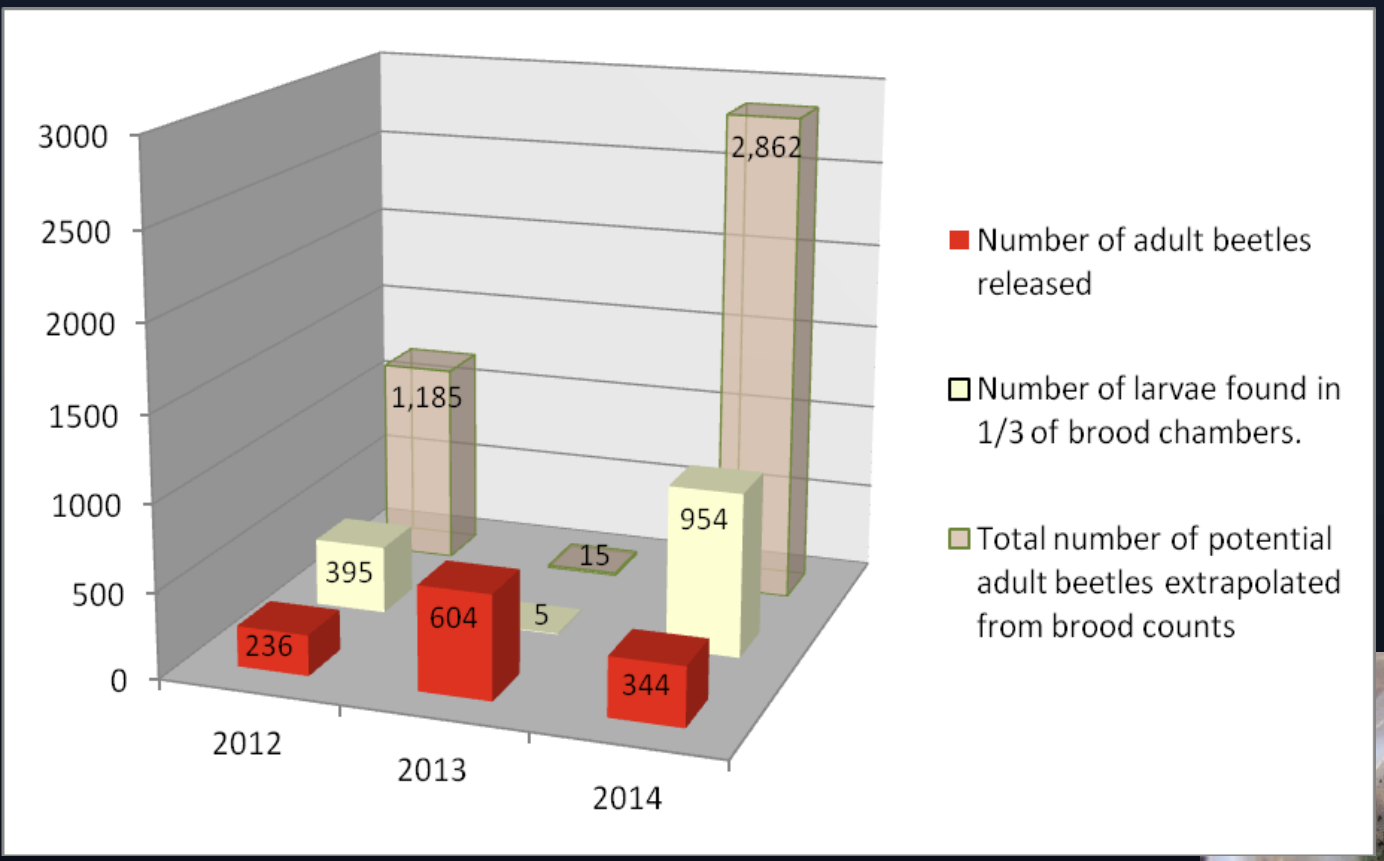
# Results


3000
2500
2000
1500
1000
500
0
2012
2013
2014
236
395
1,185
604
5
15
344
954
2,862
Number of adult beetles released
Number of larvae found in 1/3 of brood chambers.
Total number of potential adult beetles extrapolated from brood counts

# Missouri reintro 2013

- ***"The best laid schemes o' Mice an' Men, Gang aft agley…"***

**-Robert Burns**

**From the poem "To a Mouse"**

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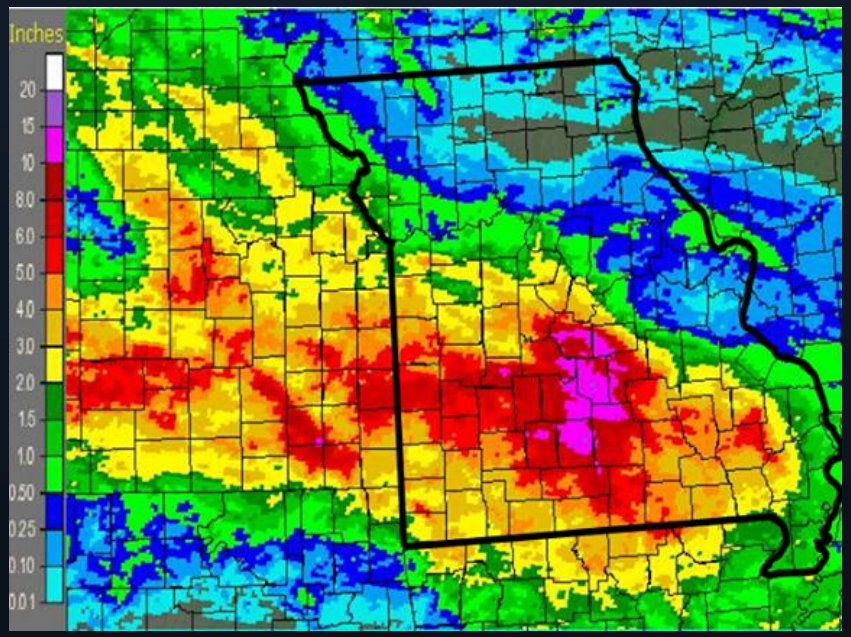
# Missouri reintro 2013

- On June 5, the day after the reintroduction of American Burying Beetles to Wah' Kon-Tah Prairie, an unexpectedly intense rainstorm stalled over the release site…

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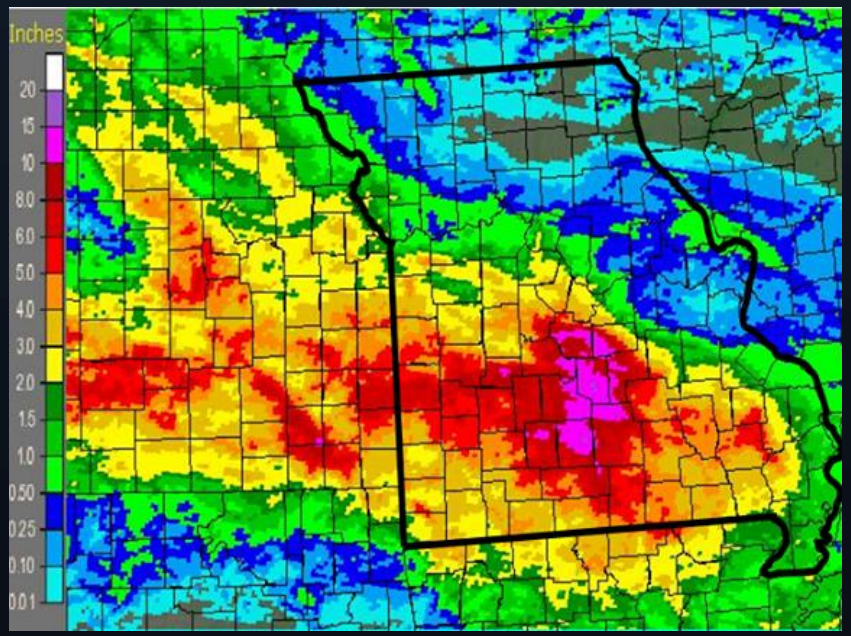
# Missouri reintro 2013

- …dropping 1.35 inches of rain  in a few short hours on already saturated ground.

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# Missouri reintro 2013

- One inch of rain amounts to 270,000 gallons falling over each 10 acre area

So…

In a little over 4 hours, more than 145,076,400 gallons of water fell on Wah' Kon-Tah Prairie

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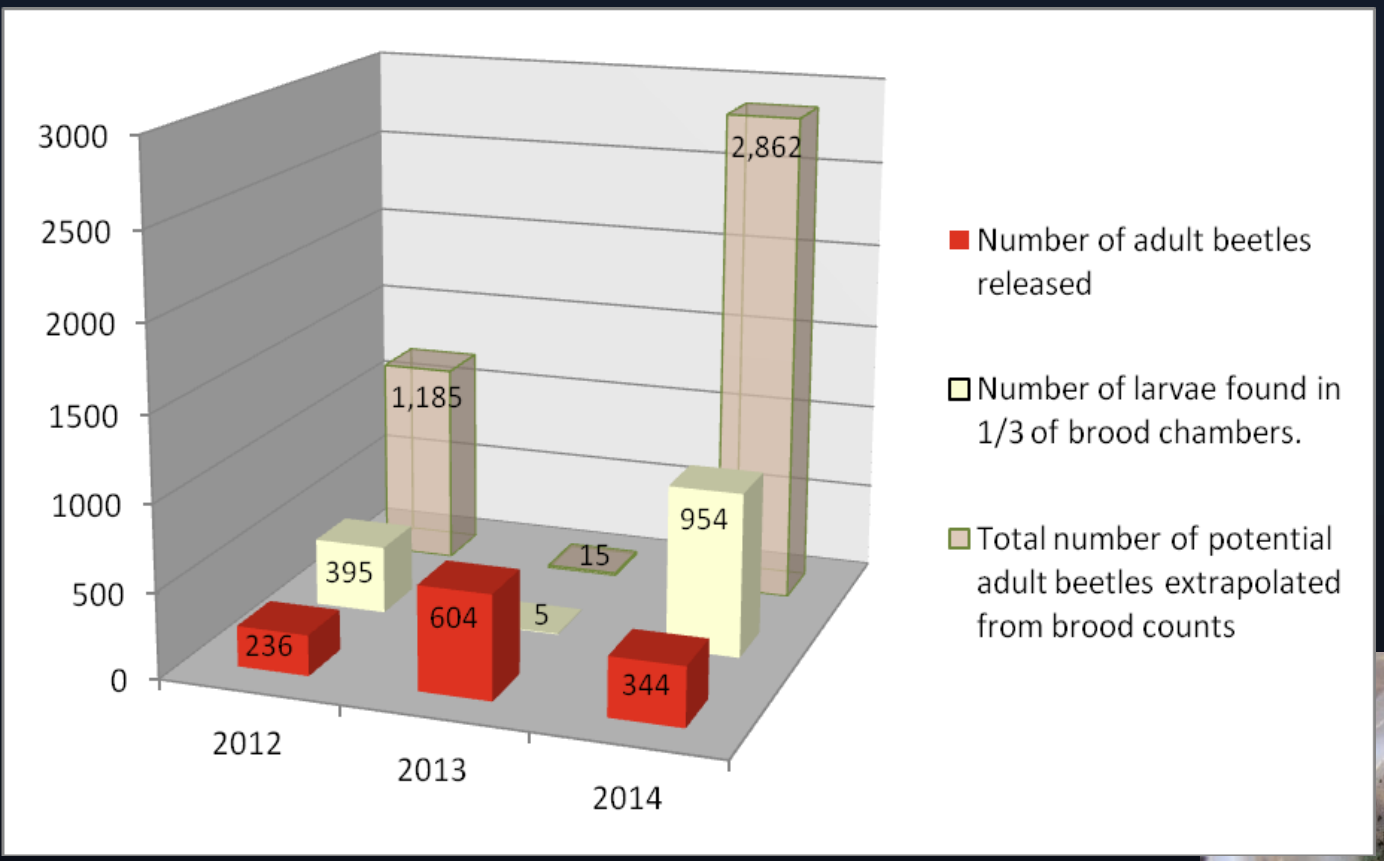
# Results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Number of adult beetles released
Number of larvae found in 1/3 of brood chambers.
Total number of potential adult beetles extrapolated from brood counts

# Reintro method…

- 66 days after reintro. We start surveying again for newly emerged beetles.
- Biometrics are recorded.
- Elytra are notched according to location found and recorded.
- Beetles are often provisioned and released after survey period is complete.

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# Reintro method…

- Surveys then resume each May to assess overwintering success.
- Biometrics and notching are noted.


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# Reintro method

- **Following 2012 release:**
  - Autumn survey captured **2** un-notched ABBs
  - Spring survey (2013) captured **1** un-notched ABB
- **Following 2013 release:**
  - Autumn survey captured **15** un-notched ABBs
  - Spring survey (2014) captured **3** un-notched ABB
- **Following 2014 release:**
  - Autumn survey captured **36** un-notched ABBs

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# Goals of ABB Genetic Project

**By using 'microsatellite region' diversity within/between ABB populations and individuals we hope to:**

- Determine genetically how distinct the existing natural populations may be
- Determine how similar or different individual beetles are within captive populations

**This information could:**

- determine if natural populations will need to be considered separate populations
- guide captive breeding programs on how to supplement populations


(A) Saint Louis Zoo *St. Louis, MO* **50**

(B) University of Nebraska at Kearny **11**

(C) Roger Williams Zoo *Providence, RI* **51**

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# Genetic Approaches Used

**Table 1.** Details for 9 polymorphic microsatellite loci developed for *Nicrophorus americanus*. The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is *N*; *k* is number of alleles observed; $H_o$ and $H_e$ are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus, and TD refers to the touchdown protocol used for pcr.

| Locus | Primer Sequence 5' --> 3' | Repeat motif | Size (bp) | N | *K* | $H_o$ | $H_e$ | PI | TD |
|---|---|---|---|---|---|---|---|---|---|
| Niam6† | F: *CCTTCCGTCGATCAGACACC<br>R: ACGTGTGCCCTCACGTCG | AAC | 201-213 | 22 | 4 | 0.136 | 0.662 | 0.178 | TD65 |
| Niam10 | F: *GACTCAATGTCTAAATCGCTGTCG<br>R: CAGCTACACCCACCATGACG | TCG | 177 (monomorphic) | 23 | 1 | 0.00 | 0.00 | 1.00 | TD65 |
| Niam15 | F: *TACAAATTCATTGGAGGCGG<br>R: CGCAATGATTTCGAACTTTCC | TGC | 106-118 | 24 | 5 | 0.375 | 0.511 | 0.28 | TD65 |
| Niam16† | F: *GGCGTCGCTTCAGTATTTGC<br>R: CGGTACCAGCAATTGCACC | AAC | 140-194 | 22 | 8 | 0.500 | 0.814 | 0.059 | TD65 |
| Niam30 | F: *TCCAACATTCTATTTGCGGC<br>R: GTTCACTCAACTGCGGATGC | TTC | 253-265 | 19 | 5 | 0.684 | 0.771 | 0.089 | TD65 |
| Niam42 | F: * GGGAGTTCTGGAACTGAGAGC<br>R: TGTTGTCGTCGTAGTCGTCG | TGC | 170-182 | 21 | 5 | 0.333 | 0.585 | 0.23 | TD65 |
| Niam2 | F: *TTTAGACCGGTTGGTGATGC<br>R: AAAGTCGGGAAGGAACAGCC | ATC | 167-176 | 23 | 2 | 0.043 | 0.043 | 0.92 | TD65 |
| Niam4† | F: *TGAATAAGTTAACTGCTCCGGC<br>R: AATTCCTTCCTCCTCCGC | TCC | 222-234 | 20 | 5 | 0.300 | 0.530 | 0.26 | TD65 |
| Niam32† | F: *TGAAATTGCTTGTGCATAGGG<br>R: TCAGCTGGAATCAGAAGGGC | AAAT | 132-136 | 20 | 2 | 0.300 | 0.480 | 0.39 | TD65 |
| Niam35 | F: *AAAGGAAGAAGAAAGAGGAGGC<br>R: TCGTTCGCATTATGTTCGC | TTC | 151-166 | 20 | 5 | 0.700 | 0.596 | 0.24 | TD65 |

* indicates CAG tag (5'- CAGTCGGGCGTCATCA -3') label;
† indicates significant deviations from Hardy-Weinberg expectations after Bonferroni corrections.

- >48 microsatellite regions located and screened using NextGeneration Sequencing
- 9 polymorphic regions found!
- Data generated will be analyzed using population genetic software such as STRUCTURE and statistical methods

Developed at Savannah River Ecology Laboratory

Funding from Saint Louis Zoo WildCare Institute®

**Preliminary Results expected December 2014**
**Final Results and Publication expected 2015**

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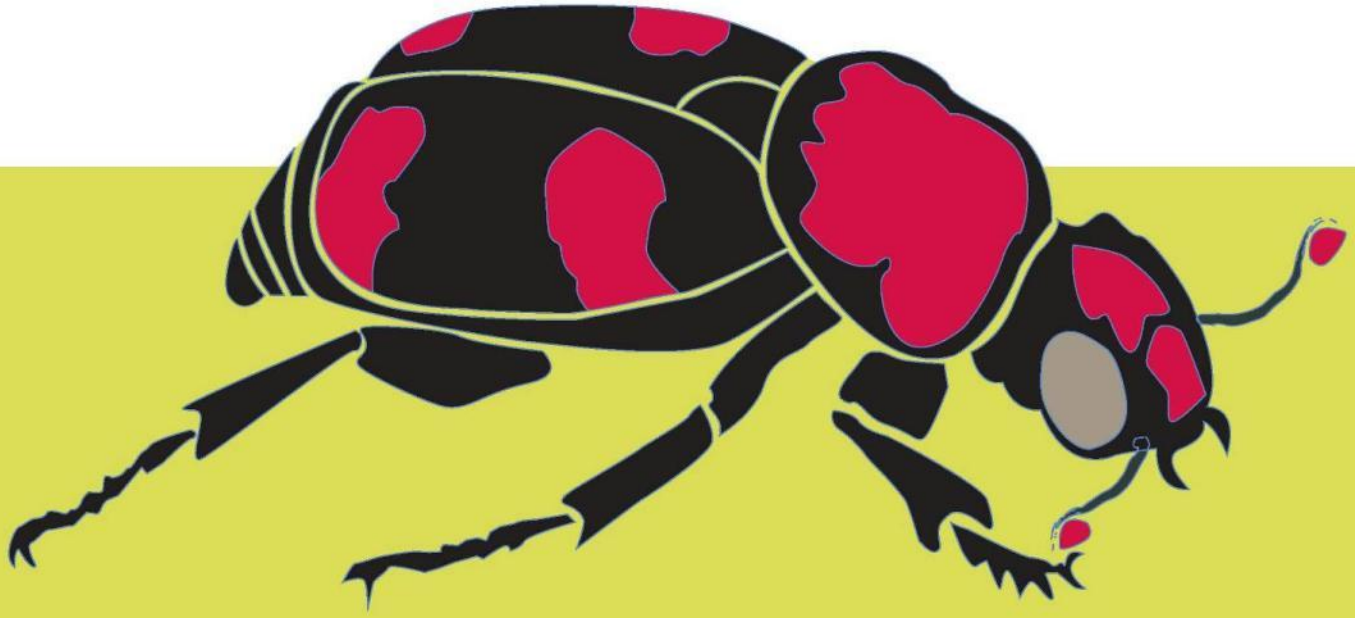
# Questions?



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